REMARKS

Of claims 1-68 which were contained in the original application, claims 37-65 which were withdrawn as being drawn to non-elected inventions are now cancelled. Claims 1-4, 10-22, 28-36 and 66-68, which are drawn to a method of treating atherosclerosis in a mammal lysosomal acid lipase, remain under consideration.

In addition to this response, Applicants have attached a Declaration under 37 CFR 1.132 from David Hui, Ph.D. The Examiner is reminded of the duty to consider such evidence. An expert declaration alone may be sufficient to satisfy the Applicants' burden with regard to the Examiner's obviousness rejection and such evidence must be considered, In re Piasecki, 745 F.2d 1468, 1471, 223 USPQ 785, 787 (Fed. Cir. 1984), and may be sufficient to overcome a prima facie case of obviousness. Id. at 1472, 223 USPQ at 788, (quoting In re Surrey, 50 C.C.P.A. 1336, 319 F.2d 233, 235, 138 USA 67, 69 (CCPA 1963)). The Declaration clearly and unambiguously states that based upon Escary (1999), one would predict that LAL therapy would be counter-productive and would result in increased atherosclerosis similar to the HSL mice discussed by Escary. Thus, the Hui declaration asserts that Escary (1999) teaches away from the Grabowski and Du invention and that one reading Escary would not be motivated to administer LAL in order to treat atherosclerotic lesions. Applicants have also submitted an additional article that teaches away from the use of LAL to treat atherosclerotic lesions (see Supplemental IDS; "Letting Lipids Go," Curr. Opinions in Lipidology, 2003, 14: 289-97). These newly submitted materials attest to the fact that current literature teaches away from the exogenous administration of LAL to treat atherosclerotic lesions. Accordingly,

based upon the Declaration and the arguments set forth below, the Examiner's obviousness rejections should be withdrawn.

Before considering the rejections in detail, the fundamental concepts of the present invention will be briefly reviewed. The present invention comprises a method to diminish and/or eliminate atherosclerotic plaques in mammals, through direct treatment of these plaques using proteins and/or polypeptides. Generally, compositions used for practicing this invention include lipid hydrolyzing proteins or polypeptides, and in particular, the protein lysosomal acid lipase (LAL). These proteins and/or polypeptides are capable of lipid removal, primarily through hydrolysis, either by a catalytic or stoichiometric process, wherein the lipid hydrolyzing protein or polypeptide targets receptors in and/or on the cell leading to uptake into the lysosome. Lipid hydrolyzing proteins, contained in a pharmaceutically acceptable carrier, may be administered either orally, parenterally, by injection, intravenous infusion, inhalation, controlled dosage release or by intraperitoneal administration in order to diminish and/or eliminate atherosclerotic plaques. The preferred method of administration is by intravenous infusion.

Rejections Under 35 U.S.C. §103(a)

The Examiner maintains his rejection of claims 1-36 and 66-68 under 35 U.S.C §103(a) as being unpatentable over Chan et al. (1986), Bond et al. (1991), Pomerantz et al. (1993), Walters et al. (1994) and Escary et al. (1998) in view of Coates et al. (1986). In addition, the Examiner argues that the Applicants' interpretation of Escary (1999), which was offered in response to the Examiner's obviousness rejections, was not wholly on point.

In repeating the §103(a) rejection, the Examiner maintains that the combined teachings of Chan et al., Bond et al., Pomerantz et al., Walters (sic) et al., and Escary et al. (1998) all provide examples where increasing LAL activity, albeit by secondary agents, reduces atherosclerosis, and, Coates et al. established that deficiencies in LAL increased the risk of developing atherosclerosis, thereby suggesting that remedying such a deficiency could constitute an effective therapy for atherosclerosis. Therefore, given the limited to non-existent success of gene therapy methods to date, the Examiner argues it would have been obvious to a person of ordinary skill in the art to increase LAL levels by direct addition of the enzyme. Applicants respectfully traverse this rejection.

As an initial matter, there is no motivation to combine the above-cited references to arrive at the present invention. The art relied on by the Examiner establishes the use of secondary agents which stimulate production of a desired enzyme. The cited references do not address the likelihood of success of direct enzyme addition. Chan et al. describe the use of prostaglandins as potential therapeutic substances for various cardiovascular diseases including atherosclerosis. Chan et al. state that atherosclerosis may result from decreases in prostacyclin formation in the blood vessel wall due to inhibition by high concentrations of lipid peroxides in the blood and that prostacyclin stimulates cholesterol ester hydrolase. Accordingly, Chan et al. maintain that prostacyclin and other related prostaglandins may be useful for the prevention of atherosclerosis since they are thought to stimulate production of CEH (cholesterol ester hydrolase) for mobilization of cholesterol. Bond et al., Pomerantz et al., and Walters et al. (*sic*) each describe the use of calcium channel blockers (antagonists) for stimulation of cholesteryl ester hydrolase activity wherein cholesteryl ester hydrolase is thought to increase clearance of accumulated

cholesterol. Again, as in the reference of Chan et al., these calcium channel blockers play a secondary role in reduction of atherosclerotic lesions in that they stimulate production of the polypeptide cholesteryl ester hydrolase, which in turn is thought to play a role in clearance of cholesterol. None of these references teaches the direct administration of an enzyme to a patient.

Escary et al. (1998) discuss the use of a *hormone*-sensitive lipase while the present invention relies on a *lysosomal* acid lipase for inhibition of atherosclerotic lesions. These two lipases function by different biochemical mechanisms within the cell. Hormone-sensitive lipase is found within the cytoplasm of the cell while lysosomal acid lipase is found within the lysosome. Although the EC numbers of the two enzymes are the same, LAL and the neutral (or hormone sensitive lipase) have completely different sequences (gene and protein) and are compartmentalized differently. Moreover, the referencing of Chan et al. (1986), Bond et al. (1991), Pomerantz et al. (1993), Walters et al. (1994) and Escary et al. (1998) in terms of enhancement of cholesteryl esterase activity is in error because all of these articles discuss non-lysosomal proteins and thus are not relevant to the present invention. This is particularly the case since the present invention (use of LAL) has shown a positive effect and Escary (1999) has shown the opposite effect by HSL increases. Thus the two enzymes are different and given such differences, one enzyme cannot substitute one for the other and expect the same biochemical behavior.

Finally, Coates et al. teach that low acid lipase activity may represent an independent risk factor for the development of premature atherosclerosis due to inherited deficiencies in this enzyme. Coates et al. does not teach that direct administration of LAL would alleviate such a deficiency, nor does Coates suggest or motivate one skilled in the art to administer LAL in order to treat such a deficiency.

Coates merely suggests that there may be a *familial link* for low acid lipase activity due to an inherited allele which confers low enzyme activity. Coates et al. does not teach or suggest any methods of treatment for low LAL but rather is focused on a genetic link to low LAL levels.

Accordingly, one would not look to Chan et al., Bond et al., Pomerantz et al., Walters (sic) et al., and Escary et al. (1998) in view of Coates to support the proposition that direct (exogenous) administration of LAL would be expected to successfully clear atherosclerotic lesions, since Chan et al., Bond et al., Pomerantz et al., Walters (sic) et al., and Escary et al. (1998) are directed only to indirect production of CEH and Coates et al. discusses familial links to low LAL levels but does not teach or suggest any methods of remedying the problem.

Also, in the previous office action, the Examiner makes the point that it is known in the art to use both direct addition of an enzyme or to use compounds that induce production of the enzyme for treating an enzyme deficiency or increasing enzyme activity. In support of this, the Examiner states that thrombotic events are often treated by "the addition of clot dissolving enzymes such as streptokinase, protein C and TPA" and that a person of ordinary skill in the art would be reasonably apprised of alternative methods of increasing activity. What the Examiner is, in essence, saying is that there are a limited number of routes to provide enzymes to the body and it would be obvious to try one of them with LAL to se if it works. Certainly, none of the references cited by the Examiner gives any basis to believe that exogenous administration of LAL would work. However, "obvious to try" cannot be the basis for an obviousness rejection. Enzymes such as streptokinase and TPA are administered in a targeted fashion and are only administered during a very discrete

period of time (generally within 30-60 minutes of the heart attack) in order to successfully dissolve the blood clot.

It is well known in the art that enzyme replacement therapy generally does not work well because of complications in the neural delivery of exogenous enzymes, cellular uptake of exogenous enzymes, and the necessary modification of enzymes to protect against degradation (see "Enzyme-Replacement Therapy: Problems and Prospects," Rademaker, B.; Raber, J.; Pharm. Weekbl. Sci., vol. 11, Oct 20 1989, pp. 137-145). Furthermore, enzyme replacement by means of I.V. injection of highly purified enzyme is complicated by a host of problems. Exogenously infused enzymes must reach the intracellular site where catalytic activity is required; in the process the enzyme must be protected from degradation by proteolytic enzymes as well as attack from endogenously produced antibodies (see "Genetic Engineering, Enzyme Immobilization, and Transplantation," Updike, S. J.; American Journal of Pharmaceutical Education (USA), vol. 36, Dec. 1972, pp. 718-722). Also, while preclinical studies in animal models have shown the efficacy of enzyme replacement therapy for the treatment of lysosomal storage diseases, these studies have also identified limitations of enzyme replacement therapy, including the inability to deliver exogenous enzymes efficiently (see "Enzyme Replacement and Enhancement Therapies: Lessons from Lysosomal Disorders;" Desnick, R. J.; Schuchman, E. H.; Nat. Rev. Genet., vol. 3, no. 12, pp. 954-966, Dec. 2002). Copies of these references are attached.

The LAL therapy described in the present application is not administered upon the occurrence of a discrete event but rather is administered over a much longer period of time (months–years) in order to treat a condition that has taken years to develop and be correctly diagnosed. Further, there are differences in degree of

response and in differential tissue response due to distribution of the enzyme when the enzyme is administered exogenously. In fact, Dr. Hui, in his declaration states that "[i]t is well-known in the art that generally, direct enzyme administration (i.e., exogenous administration) does not work well because of rapid clearance from circulation" (see paragraph 9 of the attached declaration). Therefore, due to the inherent unpredictability in the biochemical area, one cannot state, with any degree of certainty, whether exogenous administration of an enzyme would have the same desired effect as either indirect or endogenous production of that same enzyme.

Further, the Examiner maintains that since Chan et al., Bond et al., Pomerantz et al., Walters (sic) et al., and Escary et al. (1998) establish that mechanisms that increase levels of LAL activity reduce atherosclerotic plaques by enhancing net cleavage of cholesterol esters, a person of ordinary skill in the art would have been motivated to directly administer LAL to treat atherosclerosis. The Applicants point out that such is not the case and cite two references that teach away from this assertion.

First, the publication by Escary (Escary et al., "Paradoxical Effect on Atherosclerosis of Hormone-Sensitive Lipase Overexpression in Macrophages," Journal of Lipid Research, vol. 40, pp. 397-404, 1999, see Supplemental IDS), discloses the surprising finding that macrophage-specific HSL (hormone-sensitive lipase) overexpression leads to **greater** susceptibility to developing atherosclerosis. This is exactly the opposite effect observed in the present invention – which is that exogenous administration of lysosomal acid lipase produces a **decrease** in atherosclerotic lesions.

In the previous office action, the Examiner maintained that the Applicants' interpretation of Esacry (1999) was not correct. The Applicants disagree with this

contention. Focusing on the discussion on page 403 (beginning on 402), the result of overexpression of HSL in macrophages leads to increased severity of the atherosclerotic lesions. This is exactly the opposite result as predicted when based on the results in RAW macrophages and from the flux measurements in which cholesteryl esters are hydrolyzed more rapidly than without overexpression. These results demonstrate that any method of increasing this enzyme in macrophages will increase the severity of atherosclerosis.

Moreover, the attached declaration of Dr. David Hui clearly and unambiguously states the Examiner's interpretation of Escary (1999) is not wholly on point and that ACAT does not come into the picture here, because if one inhibits ACAT, this will induce cell toxicity, which is bad. It further states that based upon Escary (1999), for patients with low HDL, one would predict that LAL therapy would be counter-productive and would result in increased atherosclerosis. Dr. Hui concludes that, Escary (1999) teaches away from the Grabowski and Du invention in that one reading Escary would not be motivated to administer an exogenous LAL in order to treat atherosclerotic lesions.

Applicants also cite a second article that teaches away from the use of LAL to treat atherosclerotic lesions (see Supplemental IDS; "Letting Lipids Go," Haemmerle et al, Curr. Opinions in Lipidology, 2003, 14: 289-97). Specifically, on page 292, right column, second paragraph, the authors state that "HSL-deficiency mice have an athero-protective lipoprotein profile with low levels of apolipoprotein B containing lipoproteins, low plasma triglyceride levels and increased HDL-cholesterol concentrations." Thus, a deficiency in HSL activity in mice gives rise to anti-atherosclerotic lipoprotein profiles and suggests that HSL has pro-atherosclerotic

roles. Accordingly, Haemmerle teaches away from using the HSL gene or protein as an anti-atherosclerotic agent.

In addition, on page 293, left column, first paragraph of the same article, the authors state "[i]n HSL-knockout mice, unchanged neutral cholesteryl ester hydrolyase activities and HSL-independent cholesteryl ester hydrolysis in peritoneal macrophages provided evidence against an important role of HSL as cholesteryl ester hydrolase in macrophages." It is well known that macrophages are a major cellular component in atherosclerotic lesions. However, as Haemmerle points out, the role of HSL as a cholesteryl ester hydrolase in macrophages is not supported by the evidence. Therefore, Haemmerle teaches away from using HSL as a cholesteryl ester hydrolysis reagent.

In summary, none of these references, taken together, teach or suggest the present invention and the Examiner's rejections under 35 U.S.C. §103(a) have been overcome and should be withdrawn. Accordingly, the present application is in form for allowance and early reconsideration and allowance of the claims, as currently pending, is earnestly solicited.

Respectfully submitted,

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